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Biochemistry

DNA unwinding component of the nonhistone chromatin proteins

(rat liver/DNA affinity chromatography/non-species-specific interaction/preferential A-T binding)

TERRY L. THOMAS* AND GORDHAN L. PATEL†

Department of Zoology, University of Georgia, Athens, Ga. 30602

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ABSTRACT **A subclass of nonhistone chromatin proteins from rat liver, previously shown to exhibit high affinity for DNA, has been fractionated by single-stranded DNA-agarose affinity chromatography. The protein fraction that bound to DNA-agarose in 0.19 M NaCl-buffer and was eluted with 2 M NaCl-buffer is enriched for a protein component of approximately 20,000 daltons and exhibits preferential binding to denatured DNA. This nonhistone protein fraction specific for single strands binds to DNA in a non-species-specific manner, and causes helix-coil transition of synthetic poly[d(A-T)·d(A-T)] at 25°, as indicated by the increase in absorbance of ultraviolet light at 260 nm. The observed hyperchromicity does not result from any nuclease activity in the protein fraction, because addition of Mg^{+2} results in partial hypochromic shift, and the protein/DNA complex is retained by nitrocellulose filters.**

Protein/DNA interactions are clearly important in the structure and function of the eukaryotic chromatin. Interactions of histones with DNA have been extensively analyzed and, in recent years, have been shown to give rise to the subunit structure of the chromatin (1–5). The role of the nonhistone chromatin proteins (NHCP) in the chromatin is also a subject of much current interest. However, the NHCP are a heterogeneous class of proteins that have long resisted fractionation and characterization. Recently, the DNA binding properties of the NHCP have been exploited to fractionate and characterize them, and to gain insight into their possible biological roles (6–17).

In our laboratory, we have isolated a distinct subclass of the NHCP, designated APNH (acidic proteins with affinity for nucleohistone) (18), and have studied its *in vitro* DNA binding properties. We have demonstrated that APNH binds to DNA optimally at physiological conditions (9–11). Equilibrium and kinetic competition experiments indicated that this protein fraction interacts preferentially with A-T rich and/or single-stranded DNA, which suggested that APNH might affect the helix-coil transition of DNA (11). In this communication, we report on the isolation of a component of the APNH that unwinds synthetic poly[d(A-T)·d(A-T)] at room temperature.

MATERIALS AND METHODS

Preparation of DNA. The preparation and purification of phage T7 DNA labeled with [^{3}H]thymidine unlabeled rat liver nuclear DNA, and commercial calf thymus DNA (Worthington Biochemicals) was as described previously (11). The DNAs designated as double stranded were digested with S1 nuclease and purified by banding in CsCl (19). Alternating poly[d(A-T)·d(A-T)], procured from Miles Laboratories, was dissolved in 0.05 M KCl, 0.01 M Tris·HCl, 0.001 M Na_2EDTA (pH 7.5) and extensively dialyzed against the same buffer. Other DNAs were dissolved in 0.19 M NaCl, 0.01 M Tris·HCl, 1 mM Na_2EDTA (pH 7.5). All DNAs were stored at 4° over $CHCl_3$.

Preparation of Protein. The APNH subfraction of the rat liver nonhistone chromatin proteins was isolated as described elsewhere (11). This subfraction contains about 10% of the total NHCP and precipitates with the deoxynucleohistone when chromatin solution in buffered 2 M NaCl and 5 M urea is dialyzed against 13 volumes of distilled water. It is separated from histones and DNA by chromatography on hydroxylapatite (18). All solvents used for protein extractions contained 0.1 mM phenylmethylsulfonyl fluoride to inhibit proteases. Proteins were concentrated by ultrafiltration on PM-10 membranes (Amicon Corp.)

Isolation of the DNA Unwinding Nonhistone Protein Component. Te DNA-unwinding component of the APNH was isolated by affinity chromatography on single-stranded DNA-agarose. A solution of purified calf thymus DNA (1–2 mg/ml) was mixed with sufficient powdered agarose (Bio-Rad Laboratories) to give a final concentration of 1%, and heated at 100° for 15 min. The solution was rapidly cooled to 0° and "cured" at room temperature for 1–2 hr. Under normal conditions, 70–95% of the DNA is irreversibly trapped in the agarose matrix. Essentially all of the DNA in the agarose matrix is accessible to protein interaction as judged by its sensitivity to pancreatic DNase (19). The single-stranded DNA-agarose gel was disrupted by passing through a tissue press (Harvard Apparatus), extensively washed with 0.19 M NaCl, 5 M urea, 0.01 M Tris·HCl, 0.001 M Na_2EDTA (pH 7.5), and packed into a column. The column was further washed with this solvent until the effluent concentration of nucleic acid was undetectable. The washed DNA-agarose contained 200–500 μg of DNA per ml packed bed volume. A preparation of APNH, extensively dialyzed against this starting buffer, was applied to the column at a DNA/protein ratio greater than 10, and the unbound protein fraction was washed through the column with the starting buffer. The protein fraction bound to the DNA-matrix was eluted with 2 M NaCl, 5 M urea, 0.01 M Tris·HCl, 0.001 M EDTA (pH 7.5), and passed through DEAE-Sephadex (Pharmacia Fine Chemicals) in 0.3 M NaCl, 8 M urea, 0.005 M phosphate buffer (pH 8) to remove contaminating nucleic acid (11). All proteins were concentrated, dialyzed against appropriate buffers, and stored at 4°. Agarose matrix lacking DNA failed to bind any protein under these chromatographic conditions.

Nitrocellulose Filtration Assay for DNA/Protein Interaction. DNA/protein interaction was assayed by nitrocellulose filtration as described previously (11). In this assay, DNA/protein complexes are retained on the filter, while free DNA passes through. The extent of interaction is expressed either as the total amount of ^{3}H-labeled DNA or the fraction (R) of the initial input ^{3}H-labeled DNA retained on the filter as a function of initial protein concentration.

Abbreviations: NHCP, nonhistone chromatin proteins; APNH, acidic proteins with high affinity for DNA; t_m, temperature at which strand separation occurs.

* Present address: Division of Biology, California Institute of Technology, Pasadena, Calif. 91125.

† To whom reprint requests should be addressed.

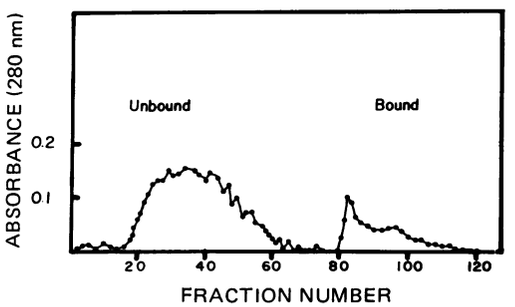

FIG. 1. Preparative DNA-agarose chromatography of APNH. APNH in 0.19 M NaCl, 5 M urea, 0.01 M Tris·HCl, 0.01 M EDTA (pH 7.5) was applied to a single-stranded DNA-agarose column at a DNA/protein ratio greater than ten. "Unbound" protein was washed from the column in the same buffer. The "bound" protein was eluted from the column with the above buffer containing 2 M NaCl. The A_{280} of each fraction was used as a measure of protein content. Protein containing fractions were pooled and the actual protein content was determined colorimetrically. Recovery from this column was 90%.

Assay of Protein Facilitated Helix-Coil Transition of Poly[d(A-T)·d(A-T)]. A Beckman Acta III double-beam spectrophotometer, equipped with temperature regulated sample compartment, automatic sample changer, and recorder was used to study the protein facilitated helix-coil transition of poly[d(A-T)·d(A-T)]. A stoppered and divided cuvette with two compartments (0.45 cm light path each) in series, separated by a partial partition, was employed to establish a preinteraction base line. Poly[d(A-T)·d(A-T)] in 0.05 M KCl, 0.01 M Tris·HCl (pH 7.5), 0.001 M EDTA was placed in one compartment and an equal volume of the protein, in the same solvent but containing 5 M urea, was placed in the other. A reference cuvette with a mixture of the two solvents in equal proportions contained a micro thermistor to monitor the temperature which was regulated at 25°. After establishing a stable base line at 260 nm, the protein and poly[d(A-T)·d(A-T)] were quickly mixed by inverting the sample cuvette several times, and the absorbance was recorded as a function of time.

Analytical Methods. DNA concentrations were determined by UV absorption at 260 nm. Protein concentrations were determined by UV absorption at 280 and 260 nm (20) and by the Lowry *et al.* method (21). Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of proteins was as described by MacGillivray *et al.* (22). Densitometric scans of the gels were recorded in a Beckman Acta III spectrophotometer. [^{3}H]DNA retained on nitrocellulose filters was determined by liquid scintillation assay in a toluene based mixture.

RESULTS

Enrichment of the single-stranded DNA specific component of NHCP

We have previously demonstrated that APNH, a distinct subfraction of the nonhistone chromatin proteins, exhibits high affinity for single strand and A-T rich DNA (11). In general, proteins or ligands which preferentially bind to the coil form of DNA as opposed to the duplex form, destabilize the duplex and lower its melting temperature (23). If such proteins preferentially bind A-T rich regions, A-T duplexes will be preferentially destabilized. From these considerations, we predicted that APNH, upon interaction with native DNA, would lower the t_m (the temperature at which strand separation occurs) of A-T rich regions of DNA. Preliminary thermal denaturation experiments with poly[d(A-T)·d(A-T)]/APNH complexes indeed suggested a substantial depression of t_m of this synthetic DNA. Although these results were complicated by light scattering problems from protein precipitation at high temperatures, they encouraged us to isolate the single-stranded DNA specific component of APNH.

In fractionation of APNH by single-stranded DNA-affinity chromatography, the protein sample was applied to the DNA-agarose column in 0.19 M NaCl, 5 M urea, 0.01 M Tris·HCl (pH 7.5), 0.001 M EDTA. Urea was required to maintain the APNH in soluble form (18). Employing the nitrocellulose filtration assay, we have measured the relative affinity of APNH for DNA at various concentrations of urea; 5 M urea decreased the binding efficiency of APNH to 20–25% of the level in the absence of urea (19). This is consistent with the results of DNA-agarose chromatography in urea (Fig. 1). In this fractionation, 65% of the applied protein failed to bind to the column while 25% bound and could be specifically eluted from the column with increasing ionic strength. A small amount of protein resisted 2 M NaCl buffer elution and could only be eluted with sodium dodecyl sulfate and therefore is not considered in this study. When the unbound fraction was rechromatographed on a second DNA-agarose column, less than 5% of the applied protein bound to the column. In contrast, rechromatography of the bound fraction that was eluted by 2 M NaCl buffer showed that more than 90% of the applied protein rebound to the DNA-agarose matrix and was quantitatively re-eluted at high ionic strength.

Densitometric scans of sodium dodecyl sulfate-polyacrylamide electrophoresis gels of the total APNH, and its DNA-agarose "bound" and "unbound" subfractions shown in Fig. 2 reveal that the "bound" subfraction is considerably enriched for a single low molecular weight protein species. The R_F of this component is indicated on the scans of the total APNH and its unbound subfraction by an arrow. This component has an approximate molecular weight of 20,000.

DNA binding properties of the APNH proteins

The DNA binding properties of the "bound" and "unbound" protein fractions from DNA-agarose chromatography were assayed by the nitrocellulose filtration method. Because the interaction of APNH with DNA is not species specific (11, 19) we used [^{3}H]DNA of phage T7 for the binding analysis. Two types of experiments were employed to evaluate the binding characteristics of the protein fractions. In the first experiment, a fixed number of DNA sites was saturated with increasing amounts of protein. Fig. 3A shows that the "bound" fraction interacts with the DNA very efficiently while the "unbound" fraction interacted minimally; more than 90% of the input DNA was calculated to be in a DNA/protein complex at saturation with the "bound" fraction.

The preferential affinity of the "bound" protein fraction for single stranded DNA was verified by an equilibrium competition experiment. Fixed quantities of [^{3}H]DNA and protein were reacted in the presence of increasing amounts of competing unlabeled DNA. As the concentration of the unlabeled DNA increases, less [^{3}H]DNA is retained on the nitrocellulose filter, because more of the protein is bound to the competing DNA. The concentration of the competing DNA at which 50% of the input [^{3}H]DNA is bound by protein is defined as $D_{1/2}$. The $D_{1/2}$ varies inversely with an association equilibrium constant, K_a. Therefore, a lower $D_{1/2}$ for a given competing DNA reflects a higher equilibrium constant for that DNA. In the experiment presented in Fig. 3B, single- and double-stranded DNA from rat liver was used as the competitor. It is evident that the single-stranded DNA is a much more effective competitor than double-stranded DNA, as reflected by their

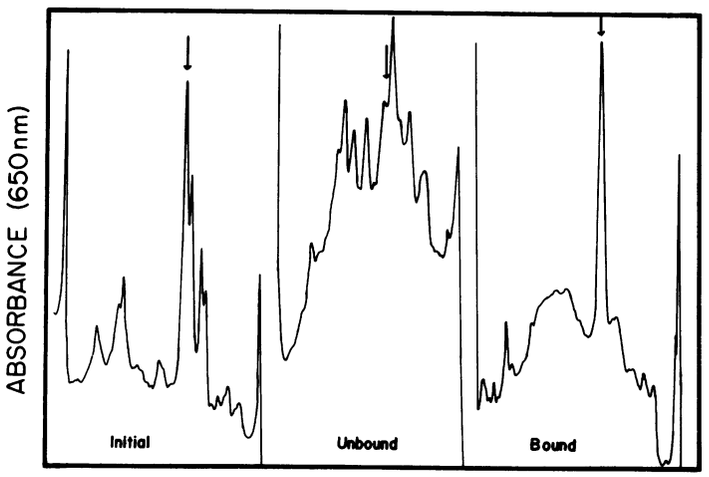

FIG. 2. Sodium dodecyl sulfate/gel electrophoresis of proteins eluted from preparative DNA-agarose column. Protein fractions eluted from the preparative DNA-agarose column (Fig. 1) and the initial APNH applied to the column were subjected to sodium dodecyl sulfate gel electrophoresis. Gels were strained in Buffalo Black, destained, and scanned at 650 nm. Electrophoresis was from left to right. The vertical arrows (↓) indicate R_F = 0.64, corresponding approximately to 20,000 daltons.

respective $D_{1/2}$s. The $D_{1/2}$s for single- and double-stranded DNAs were 0.024 μg/ml and 0.18 μg/ml, respectively.

Effect of the single-stranded DNA "bound" fraction of APNH on the helix-coil transition of DNA

The effect of the "bound" protein fraction on the helix-coil transition of DNA was determined by studying protein facilitated hyperchromicity of the poly[d(A-T)·d(A-T)]. The use of a cuvette with two compartments in series to keep the protein and DNA physically separated allowed us to establish a stable baseline for the absorbance of the sum of the two components in the same cell. If the components of the two compartments

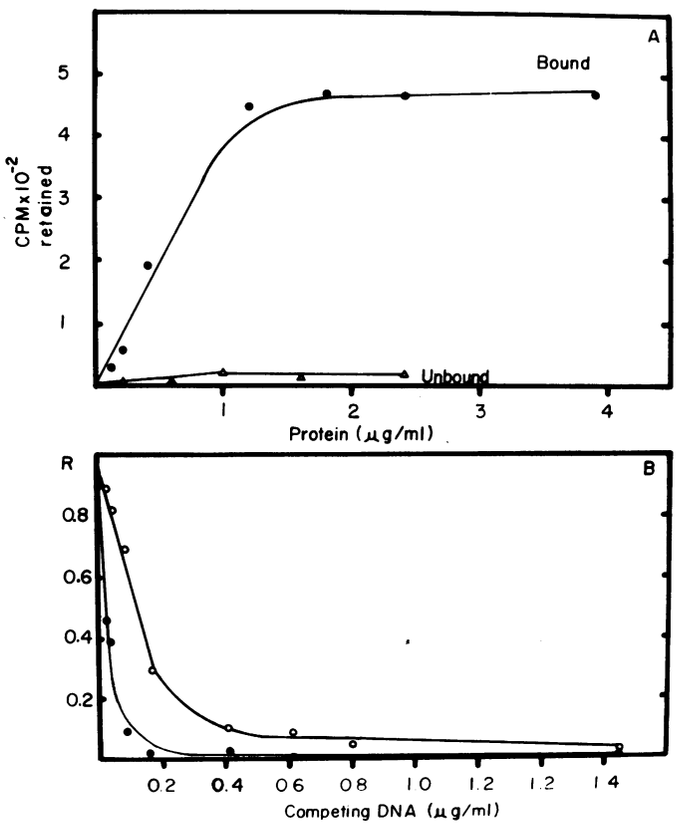

FIG. 3. DNA binding properties of fractionated APNH. (A) Saturation of T7 DNA binding sites with fractionated APNH. Increasing amounts of "bound" (●) and "unbound" (△) protein fractions from the preparative DNA-agarose column (Fig. 1) were reacted with T7 [^{3}H]DNA (0.08 μg/ml) in a final volume of 3.1 ml containing 0.19 M NaCl, 0.01 M Tris·HCl, 0.001 M EDTA (pH 7.5) plus 5% dimethyl sulfoxide. Following a 60 min reaction at 25°, 1 ml aliquots were filtered in triplicate on Schleicher and Schuell B6 filters. Filters were washed with 1.5 ml of the binding buffer at 0°, and the radioactive DNA retained on the filter was determined. Radioactive DNA retained in the absence of protein was subtracted from each point. (B) Equilibrium competition reactions with "bound" protein fractions and double- and single-stranded DNA. A constant amount of the "bound" protein fraction (1 μg/ml) and T7 [^{3}H]DNA (0.08 μg/ml) was reacted with increasing amounts of heat denatured (●) and native (○) rat DNA under conditions identical to those in Fig. 3A. Samples were processed as above. The fraction of radioactive DNA retained, R, is normalized to one at zero competition.

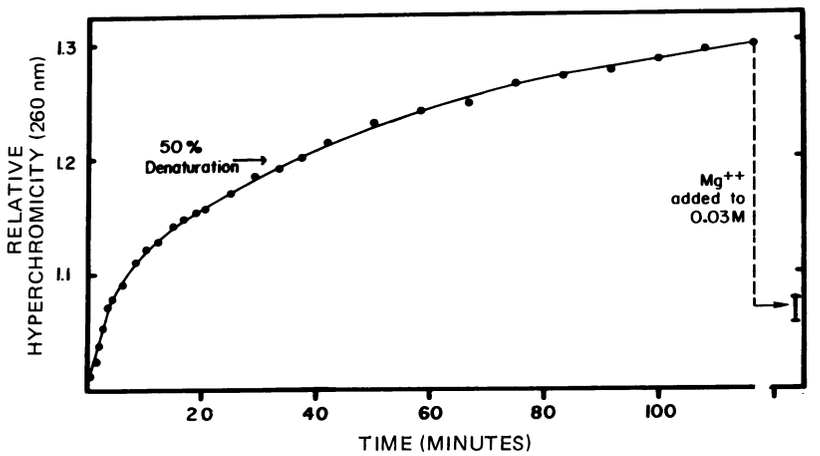

FIG. 4. Poly[d(A-T)·d(A-T)] denaturation facilitated by fractionated APNH. Eighty micrograms of the "bound" protein fraction (Fig. 1) in 1.2 ml 0.05 M KCl, 0.01 M Tris·HCl, 0.001 M EDTA (pH 7.5) plus 5 M urea and 5.5 μg of poly[d(A-T)·d(A-T)] in the same buffer but lacking urea were placed in separate compartments of a divided UV cell and were allowed to equilibrate at 25°. The two compartments were mixed by inverting the cell. The A_{260} was recorded automatically as a function of time. The initial A_{260} was 0.085. Mixing the "bound" protein fraction with the poly[d(A-T)·d(A-T)] solvent alone resulted in about 5% baseline increase. This was subtracted from all A_{260} measurements made with poly[d(A-T)·d(A-T)]. The final A_{260} attained was 0.119. The vertical bar in the figure indicates the relative A_{260} which results when $MgSO_4$ is added to the denatured poly[d(A-T)·d(A-T)] protein mixture to 0.03 M.

are noninteracting, then their mixing should not affect the absorbance at any wavelength since the pathlength for each is doubled and the concentration is reduced by one-half. Thus, the effects of mixing are negated. On the other hand, interacting components should undergo a change in absorbance upon mixing.

In our experiment two absorbance changes were observed. One small, time-independent increase (less than 5%) in A_{260} resulted when protein solution in one compartment was mixed with 0.05 M KCl, 0.01 M Tris·HCl, 0.001 M EDTA in the other. A second large time-dependent increase in A_{260} resulted when the "bound" protein fraction was mixed with poly[d(A-T)·d(A-T)]. This latter hyperchromic effect (Fig. 4) is attributed to the denaturation of the synthetic DNA by the single-stranded specific fraction of APNH. The data have been corrected for the change in A_{260} of the protein alone and normalized to a baseline of 1. Forty percent hyperchromicity was observed in this and other experiments. Total hyperchromicity of this poly[d(A-T)·d(A-T)] alone after heat denaturation in same buffer conditions was 43%. Fifty percent denaturation at 25° in the presence of the protein was effected in 38 min. This is only slightly longer than that reported for the gene 32 protein/poly[d(A-T)·d(A-T)] interaction (24). Correcting for the t_m depression due to 2.5 M urea (6.5°), the t_m of the poly[d(A-T)·d(A-T)] was 59° (25). Thus, the t_m depression effected by the single-stranded specific protein fraction of APNH is 33°–34°. This is in good agreement with the Δt_m (−35°) observed in thermal denaturation experiments with unfractionated APNH (19) and that reported for the gene 32 protein (24).

The hyperchromic effect in the above mixing experiment can be partially reversed by divalent cation. Addition of $MgSO_4$ to 0.03 M decreased the A_{260} considerably. We attribute this transition to renaturation of the poly[d(A-T)] duplex. Due to the extremely low nucleotide complexity of this synthetic DNA, this transition occurs so rapidly that no reliable kinetics of the renaturation have been recorded. However, the relative final value of the Mg^{+2} mediated hypochromicity is indicated in Fig. 4.

DISCUSSION

The role of the nonhistone proteins in the structure and function of the eukaryotic chromatin is presently a subject of much interest. Although they have been implicated in the regulation of transcription (26, 27) little is known about the molecular basis of their effect on the DNA template.

In any consideration of gene functions, unwinding of the DNA template is clearly an important step. We have shown in this study that a fraction of the nonhistone proteins, which was isolated on the basis of its affinity for single-stranded DNA, effects unwinding of a synthetic DNA at ambient temperature. This fraction, although not homogeneous, is markedly enriched for a low-molecular-weight component, which we infer to be the single-stranded specific protein. It is clearly present in the total protein applied to the DNA-agarose column but is depleted from the ("unbound") protein fraction that failed to bind to the column under the fractionation conditions (Fig. 2). It is not clear whether the remaining proteins in the fraction with the unwinding activity are also DNA binding proteins which copurified with this low-molecular-weight species or whether they are nonspecific contaminants. The effectiveness of the DNA-agarose chromatography employed in enriching for the single strand specific protein was evident from the rechromatography experiments and the DNA-binding properties of the proteins determined by an alternate assay.

As noted earlier, proteins which preferentially bind the coil form of DNA lower the t_m of the DNA duplex. If this interaction is sufficiently strong, then the t_m can be lowered into the physiological temperature range (23). Thus, in view of the single-stranded specificity of the DNA-binding protein fraction described here, its hyperchromic effect on poly[d(A-T)·d(A-T)] was not entirely unexpected. This phenomenon is best explained as protein facilitated helix-coil transition of the synthetic DNA. Two alternative explanations, however, could also account for this observation. The increase in absorbance could be a light scattering phenomenon resulting from either protein or protein/DNA aggregation following the dilution of urea from 5 to 2.5 M. We discount this possibility since the absorption spectra before and after mixing were essentially superimposable (unpublished data), except for the increased absorbance in the peak area; no significant absorbance increase below 250 nm was noted. Also, the DNA/protein complexes formed are soluble in aqueous buffers. Alternatively, a nucleolytic activity, if present in the protein preparation, could account for the observed hyperchromicity. We consider this also improbable since

more than 90% of T7 DNA mixed with the protein is bound and retained on the nitrocellulose filter. These DNA/protein complexes are stable with no loss of DNA as a function of time. Furthermore, the molecular weight of T7 DNA was not appreciably reduced when mixed with the protein (unpublished data). Finally, the reversibility of the hyperchromicity by addition of Mg^{++} strongly argues against nucleolytic activity. We conclude, therefore, that the hyperchromicity attendant on the mixing of poly[d(A-T)·d(A-T)] with the single-stranded specific protein fraction isolated by us truly reflects protein facilitated unwinding of the DNA.

Single-stranded specific DNA binding proteins, which lower the t_m of the DNA duplex are not uncommon. Such proteins have been studied in several prokaryotic systems, including that of the bacteriophage T4 (24, 28–31). So far as eukaryotes are concerned, similar proteins have been detected in *Ustilago maydis* (32), meiotic cells of *Lilium* (33), and in the calf thymus (34–36). However, in all of these instances the proteins have been isolated from total cell lysates and coul not be assigned to the cell nucleus. Indeed, in a recent elegant study of DNA-unwinding proteins from calf thymus (35, 36) Herrick and Alberts discuss that, *in vivo*, their protein preparation could well be specific for RNA. Consequently, the observations reported here are an important step towards elucidating the molecular basis of genome related activity of at least some of the nonhistone chromatin proteins.

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1. Kornberg, R. D. (1974) *Science* **184,** 868–871.
2. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4,** 281–299.
3. Sollner-Webb, B. & Felsenfeld, G. (1975) in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression,* eds. Stein, G. S. & Kleinsmith, L. J. (Academic Press, New York), pp. 213–226.
4. Thomas, J. O. & Kornberg, R. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2626–2630.
5. Shaw, B. R., Herman, T. H., Kovacic, R. T., Beaudreau, G. S. & Van Holde, K. E. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 505–509.
6. Kleinsmith, L. J. (1973) *J. Biol. Chem.* **248,** 5648–5653.
7. Teng, C. S., Teng. C. T. & Allfrey, V. G. (1971) *J. Biol. Chem.* **246,** 3597–3609.
8. Allfrey, V. G., Inoue, A. & Johnson, E. M. (1975) in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression,* eds. Stein, G. S. & Kleinsmith, L. J. (Academic Press, New York), pp. 265–300.
9. Patel, G. L. & Thomas, T. L. (1975) in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression,* eds. Stein, G. S. & Kleinsmith, L. J. (Academic Press, New York), pp. 249–264.
10. Patel, G. L. & Thomas, T. L. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2524–2528.
11. Thomas, T. L. & Patel, G. L. (1976) *Biochemistry* **15,** 1481–1489.
12. van den Broek, H. W. J., Nooden, L. D., Sevall, J. S. & Bonner, J. (1973) *Biochemistry* **12,** 229–236.
13. Sevall, J. S., Cockburn, A., Savage, M. & Bonner, J. (1975) *Biochemistry* **14,** 782–789.
14. Chiu, J. F., Wang, S., Fujitani, H. & Hnilica, L. S. (1975) *Biochemistry* **14,** 4552–4558.
15. Wang, S., Chiu, J. S., Klyszejko-Stefanowicz, L., Fujitani, H. & Hnilica, L. S. (1976) *J. Biol. Chem.* **251,** 1471–1475.
16. Kostraba, N. C., Montagna, R. A. & Wang, T. Y. (1975) *J. Biol. Chem.* **250,** 1548–1555.
17. Wang, T. Y. & Kostraba, N. C. (1975) in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression,* eds. Stein, G. S. & Kleinsmith, L. J. (Academic Press, New York), pp. 35–44.
18. Patel, G. L. (1972) *Life Sci.* **11,** 1135–1142.
19. Thomas, T. L., (1975) Ph.D. Dissertation, University of Georgia.
20. Warburg, O. & Christian, W. (1941) *Biochem. Z.* **310,** 384–421.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **13,** 265–275.
22. MacGillivray, A. J., Carroll, D. & Paul, J. (1971) *FEBS Lett.* **13,** 204–208.
23. von Hippel, P. H. & McGhee, J. D. (1972) *Annu. Rev. Biochem.* **41,** 231–300.
24. Alberts, B. M. & Frey, L. (1970) *Nature* **227,** 1313–1318.
25. Smith, M. J., Hough, B. R., Chamberlin, M. E. & Davidson, E. H. (1974) *J. Mol. Biol.* **85,** 103–126.
26. Gilmour, R. S. (1975) in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression,* eds. Stein, G. S. & Kleinsmith, L. J. (Academic Press, New York), pp. 19–33.
27. Stein, G., Stein, J., Thrall, C. & Park, W. (1975) in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression,* eds. Stein, G. S. & Kleinsmith, L. J. (Academic Press, New York), pp. 1–17.
28. Alberts, B. M., Frey, L. & Delius, H. (1972) *J. Mol. Biol.* **68,** 139–152.
29. Sigal, N., Delius, H., Kornberg, T., Gefter, M. L. & Alberts, B. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3537–3541.
30. Reuben, R. C. & Gefter, M. L. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1846–1850.
31. Reuben, R. C. & Gefter, M. L. (1974) *J. Biol. Chem.* **249,** 3843–3850.
32. Banks, G. R. & Spanos, A. (1975) *J. Mol. Biol.* **93,** 63–77.
33. Hotta, Y. & Stern, H. (1971) *Nature New Biol.* **234,** 83–86.
34. Phillips, A. P. (1968) *Biochem. Biophys. Res. Commun.* **30,** 393–399.
35. Herrick, G. & Alberts, B. (1976) *J. Biol. Chem.* **251,** 2124–2132.
36. Herrick, G. & Alberts, B. (1976) *J. Biol. Chem.* **251,** 2133–2141.